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Diagnostic utility of small fiber analysis in skin biopsies from children with chronic pain

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Abstract

Introduction: Small fiber neuropathies (SFN) are associated with a reduction in quality of life. In adults, epidermal nerve fiber density (END) analysis is recommended for the diagnosis of SFN. In children, END assessment is not often performed. We analyzed small nerve fiber innervation to elucidate the potential diagnostic role of skin biopsies in young patients with pain.

Methods: Epidermal nerve fiber density and sudomotor neurite density (SND) were assessed in skin biopsies from 26 patients aged 7 to 20 years (15 female patients) with unexplained chronic pain. The results were compared with clinical data.

Results: Epidermal nerve fiber density was abnormal in 50% and borderline in 35% of patients. An underlying medical condition was found in 42% of patients, including metabolic, autoimmune, and genetic disorders.

Discussion: Reduction of epidermal nerve fibers can be associated with treatable conditions. Therefore, the analysis of END in children with pain may help to uncover a possible cause and guide potential treatment options.

KEYWORDS

epidermal nerve fiber density, neuropathic pain, skin biopsy, small fiber, small fiber neuropathy, sudomotor neurite density, sweat gland innervation

Abbreviations: AOI, area of interest; END, epidermal nerve density; NCS, nerve conduction study; PBS, phosphate-buffered saline; PGP 9.5, protein gene product 9.5; QST, quantitative sensory testing; SFN, small fiber neuropathy; SG, sweat glands; SND, sudomotor neurite density; TRP, transient receptor potential.

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1 | INTRODUCTION

Small fiber neuropathy (SFN) is known to cause neuropathic pain in adults with distal distribution and autonomic symptoms, leading to a reduction in quality of life.[1-3] Small fiber neuropathy is characterized by damage to small unmyelinated C and thinly myelinated A δ nerve fibers. The underlying etiology of SFN is diverse and includes metabolic, toxic, autoimmune, or genetic disorders. The etiology of approximately 30% cases remains unknown.[2,4-10] Recent studies have found that the clinical spectrum of SFN is broader than initially suspected. In addition, generalized pain symptoms can be associated with small fiber degeneration, which makes the clinical diagnosis even more challenging.[7,9,11-14]

In children, the underlying etiology and symptoms of SFN might be different from those in adults. Diabetes mellitus is one of the most common etiologies of SFN in adults but is less common in younger patients.[15-17] Juvenile and adult patients with Fabry disease often present with severe neuropathic pain, making SFN the initial symptom.[18,19] Other genetic causes such as channelopathies and hereditary sensory neuropathies are associated with SFN.[10] Small fiber toxicity is described in both children and adults who have received chemotherapy.[20] In young patients with SFN, autoimmune diseases might often be the underlying cause, and appropriate therapies can reduce pain symptoms in a subset of patients.[21-23] However, SFN associated with other conditions, such as infectious, nutritional, or hereditary diseases, might occur in young patients with pain. In addition, small fiber pathology and reduction in epidermal nerve density (END) have been described in patients with fibromyalgia syndrome who present with chronic widespread pain.[24,25]

Diagnosing SFN remains a challenge. Small nerve fibers cannot be evaluated with routine tests, such as nerve conduction studies (NCS), which detect only impairment of the fast conducting A-α (motor NCS) and A-β (sensory NCS) fibers. Special electrophysiological tests, such as quantitative sensory testing (QST), are required to evaluate small fiber function. Normative values are available for children and young adolescents but are dependent on the patient's cooperation.[26] In addition, QST is not available in all pediatric clinics.

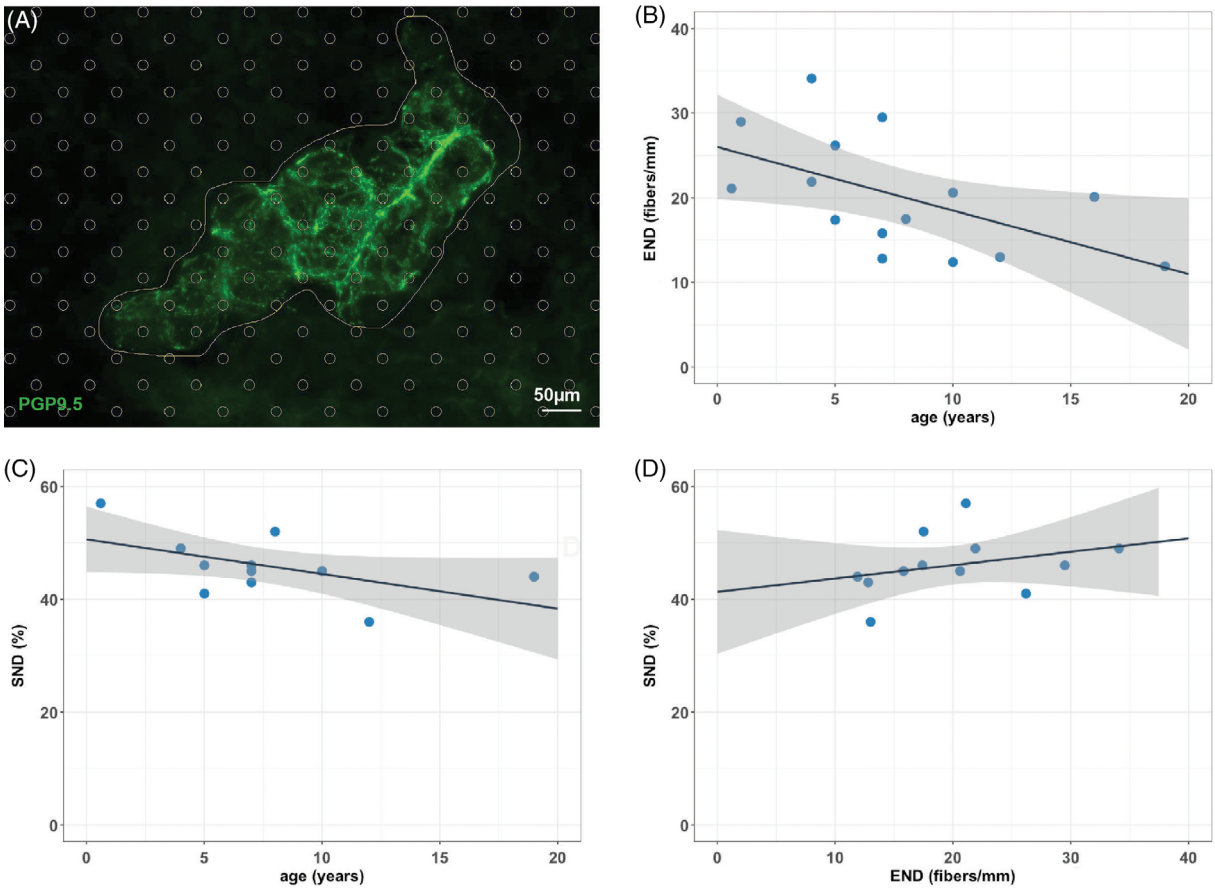

FIGURE 1 END and SND in proximal skin biopsies of the nonpain group. Analysis of sweat gland innervation with antibodies against PGP 9.5 in 50-µm sections (A). END showed a negative correlation with age (B). SND showed no correlation with age (C) and no correlation with END (D). Scale bar = 50 µm. END, epidermal nerve density; PGP 9.5, protein gene product 9.5; SND, sudomotor neurite density

TABLE 1 Summary of clinical data of pain patients

Variables	Patients, n (%)
Pain symptoms	26/26 (100)
Chronic progression, disease duration >1 year	18/25 (72)
Pain on numerical rating scale, >6/10	6/7 (86)
Diffuse distribution, legs, eyes, abdomen, back, head, joints	7/26 (27)
Distal distribution, predominant in distal legs and hands	19/26 (73)
Pain quality	20/26 (77)
Burning	6/20 (30)
Prickling or tingling	11/20 (55)
Hyperesthesia	3/20 (15)
Pressing	3/20 (15)
Course	17/26 (65)
Constant	8/17 (47)
Attacks	3/17 (18)
Intermittent	6/17 (35)
Trigger	18/26 (69)
Cold temperature	4/18 (22)
Warm temperature	2/18 (11)
Physical activity	7/18 (39)
Post infectious	2/18 (11)
Touching or pressure on trigger points	3/18 (17)
Others: stress, rest, fatigue	3/18 (17)
Sleep disturbances	11/21 (52)
Pain medication	17/22 (77)
Autonomic dysfunction	18/26 (69)
Digestion problems: nausea, constipation, dysphagia, abdominal pain, feeding problems	13/18 (72)
Incontinence: fecal and micturition	5/18 (28)
Hot flushes, dizziness, orthostatic problems	6/18 (33)
Hyperhidrosis, nocturnal sweating	3/18 (17)
Restless leg syndrome	8/26 (31)
Erythromelalgia	4/17 (24)
Muscle weakness, reduced exercise capacity, or decreased force	14/26 (54)
Fatigue	5/26 (19)
Underlying medical condition	
Idiopathic	15/26 (58)
Diabetes mellitus type 1	1/26 (4)
Autoimmune	3/26 (12)
TRPA1 c.145A > G; p.Asn49Asp	1/26 (4)
1q21.1 microdeletion	1/26 (4)
Lambert Eaton myasthenic syndrome with Xp11.22-p11.23 duplication [51]	1/26 (4)
Strümpell Lorrain syndrome	1/26 (4)
CMT	3/26 (12)
CMT1 (*PMP22* mutation)	1/26 (4)
CMT2	2/26 (8)

Abbreviations: CMT, Charcot–Marie–Tooth disease; TRPA1, transient receptor potential A1.

Analyzing epidermal nerve fibers in small skin punch biopsies is a recommended technique to allow a diagnosis of SFN in adult patients.[27] The punch biopsy procedure is minimally invasive and can be performed under local anesthesia. Several studies have analyzed END in large groups of healthy individuals, and reference values are available for female and male patients in different age groups, demonstrating a decrease in END in older individuals.[27-29] However, these studies did not include individuals younger than 20 years of age,[30,31] so interpretation of END and diagnoses of SFN in younger patients is challenging. Reports describing the use of skin biopsies for diagnosing SFN in children are not as numerous. [16,21-23] Only a few studies have analyzed END in healthy children, and reference values are not available.[30-32]

In patients with SFN, autonomic disturbances occur frequently because C fibers mediate not only pain and temperature but also autonomic function.[2,33] The involvement of autonomic nerve fibers can be evaluated by analyzing sudomotor fibers in skin biopsies.[34-38] Morphometric analyses of sweat gland innervation have been described in adults, but recommended standard values are not available.[39] Sudomotor abnormalities in children with small fiber dysfunction are frequent, but morphometric analysis of sweat gland innervation in skin biopsies has not been performed in a large cohort of children.[16,21]

In this study, we analyzed epidermal and sudomotor nerve fiber innervation to elucidate the potential diagnostic role of skin biopsies in children and adolescents with unexplained chronic pain.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

This work was approved by the ethical committee of the University of Giessen. Written informed consent was obtained from all patients or from their parents (AZ 85/16).

2.2 | Patients

Young patients with unexplained chronic pain were seen and examined at pediatric clinics in Germany and Belgium. All patients who

underwent a skin biopsy from January 2015 to May 2019 were included in the study. In all patients, extensive diagnostic testing was performed, including laboratory investigations, genetic testing, and NCS. Clinical data were collected retrospectively.

For controls (nonpain group), proximal skin biopsies were analyzed that had been obtained from 15 children and teenagers aged 6 months to 19 years who underwent a muscle biopsy for diagnostic reasons (eg, cardioskeletal myopathy or storage disorder). Performing skin biopsies in healthy children for determining standard values was deemed not suitable for ethical reasons. All patients undergoing a muscle biopsy from January 2017 to May 2019 and/or their legal guardians gave consent to excise a small longitudinal sample of skin after the incision for the muscle biopsy. None of the patients had a history of pain or complained of any clinical symptoms suggestive of small fiber pathology. Moreover, all of these patients had normal NCS and did not have any disorders known to be associated with SFN.

2.3 | Analysis of skin biopsies

Three-millimeter skin punch biopsies were taken distally, 10 cm above the lateral malleolus of the leg in all patients with pain. In the nonpain group, proximal skin biopsies were obtained from the lateral thigh. The skin biopsy analysis was performed according to European Federation of Neurological Societies guidelines.[27] Skin samples were fixed with Zamboni fixative (2% paraformaldehyde, 15% picric acid, phosphate-buffered saline [PBS]) for at least 48 hours, washed in PBS, transferred to 10% sucrose, and stored at −80°C freezer until use. From each biopsy, 50-µm thick frozen sections were stained by using a free-floating protocol. Primary antibody anti–protein gene product (PGP 9.5, 1:1000; Zytomed, Berlin, Germany) was incubated overnight at room temperature in a PBS solution containing 0.1% Triton X-100, 0.1% Tween 20, and 0.1% bovine serum albumin. The goat anti–rabbit Alexa Fluor 488 secondary antibody (1:1000; Thermo Fisher Scientific, Waltham, Massachusetts) was incubated for 3 hours at room temperature. Sections were mounted with 4′,6-diamidino-2-phenylindole (Fluorshield; Abcam, Cambridge, Massachusetts).

All samples were examined with a Leica Microsystems (Wetzlar, Germany) DM 2000 fluorescence microscope at ×400 with a Leica DFC450C camera. Morphological analysis was performed in Leica Application-Suite Version 4.7.1 and Fiji ImageJ (version 1.51n; https://fiji.sc/). The investigator (J.G.) was blinded to the specimen category during the morphologic evaluation of biopsies.

2.4 | Quantification of END

Intraepidermal nerve fibers crossing the dermal-epidermal junction were counted according to published counting recommendations.[27] At minimum, six sections were analyzed from each biopsy. Epidermal nerve fiber density was assessed as the density of the total length of the epidermis (nerve fibers/mm). Epidermal nerve density was considered abnormal when it was lower than the fifth percentile (mean of 6.1 in male patients and 8.4 in female patients) and borderline when it was between the fifth percentile and the median (mean of 10.9 in male patients and 13.5 in female patients) compared to recommended reference values for 20 to 29-year-old individuals.[32]

TABLE 2 Proximal skin biopsy analysis of nonpain group

Patient	Sex	Age at biopsy, y	END, fibers/mm	SND (%)	Etiology
NP1	M	19	11.9	44	Mitochondrial disorder
NP14	AG	16	20.1	ND	Juvenile idiopathic arthritis overlap syndrome
NP2	G	12	13	36	Cardiomyopathy
NP9	B	10	12.4	ND	Congenital myopathy
NP13	B	10	20.6	45	Becker muscular dystrophy
NP7	B	8	17.5	52	Congenital myopathy
NP4	G	7	29.5	46	Segawa disease
NP12	B	7	15.8	45	Duchenne muscular dystrophy
NP6	G	7	12.8	43	Congenital myopathy
NP10	G	5	17.4	46	Dermatomyositis
NP3	B	5	26.2	41	Motor development disorder
NP8	G	4	34.1	49	Cardiomyopathy
NP11	B	4	21.9	49	Cardiomyopathy
NP15	G	1	29	ND	Mental retardation + muscular hypotonia
NP5	IG	0.6	21.1	57	Cardiomyopathy

Abbreviations: AG, adolescent girl; B, boy; END, epidermal nerve fiber density; G, girl; IG, infant girl; M, man; ND, not done; NP, nonpain patient; SND, sudomotor neurite density.

2.5 | Quantitation of sudomotor neurite density

Sudomotor nerve density (SND) was analyzed in 50-μm-thick sections stained for the panaxonal marker PGP 9.5 by using a manual quantitation method reported by Gibbons et al.[37] Eccrine sweat glands (SG) were captured by using both in-focus and out-of-focus images to mark the area of interest (AOI) with Leica Application-Suite 4.7.0 at ×200. Sweat glands with a size <300 μm and SGs directly associated with hair follicles were excluded. At minimum, four SGs were analyzed. ImageJ was used to create a grid of circles (10 μm in diameter with a horizontal space of 50 μm and a vertical space one of 25 μm) that was merged with the in-focus image taken from the SG. Protein gene product 9.5-positive fibers crossing the circles were counted and divided by all circles, both within the AOI (Figure 1A). Sudomotor nerve density was expressed as percentage grid intercepts. No recommendations for normal values for SND are available. Therefore, we referred to Gibbons et al,[37] who reported abnormal SND in adults with diabetes mellitus type 2 (SND 20.8% ± 12.2%) and normal SND in healthy individuals (SND 40.8% ± 12.8%) and considered SND as abnormal when it was ≤28% and as borderline when it was 29% to 40%.

2.6 | Statistical analysis

Statistical analyses were performed in Prism 7 (GraphPad Software, San Diego, California) and R 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/). Initially, a Shapiro–Wilk test was performed on every variable of the data (age, END and SND for pain and nonpain as well as "progression of pain") for normal distribution of the data. We then used the Spearman rank-order correlation to compute the relationship between the variables and modeled the relationships between the variables via linear regression. We further used the Bonferroni correction to adjust the α-level from α = .05 down to α = .017 because we used END from the pain group three times in the Spearman's rank-order correlation. Two-sample t tests were used for estimations of diffuse and distal pain means of END. The values are given as mean and SD.

3 | RESULTS

3.1 | Clinical data of patients with pain

The patients with pain symptoms (n = 26) were aged 7 to 20 years (14.2 ± 3.9), with 58% female patients. The delay of diagnosis from onset of symptoms was, on average, 4 years. Most patients reported chronic distal pain triggered by various factors, including cold temperature and physical activity. An underlying medical condition that was assumed to be causally related to the patient's pain was found in 42% of patients, including metabolic, autoimmune, and genetic diseases (Table 1, Table S1).

3.2 | Analysis of END and SND in proximal biopsies from nonpain individuals

Proximal skin biopsies taken from nonpain participants showed a mean END of 20.22 fibers/mm (11.9–34.1). The highest number was

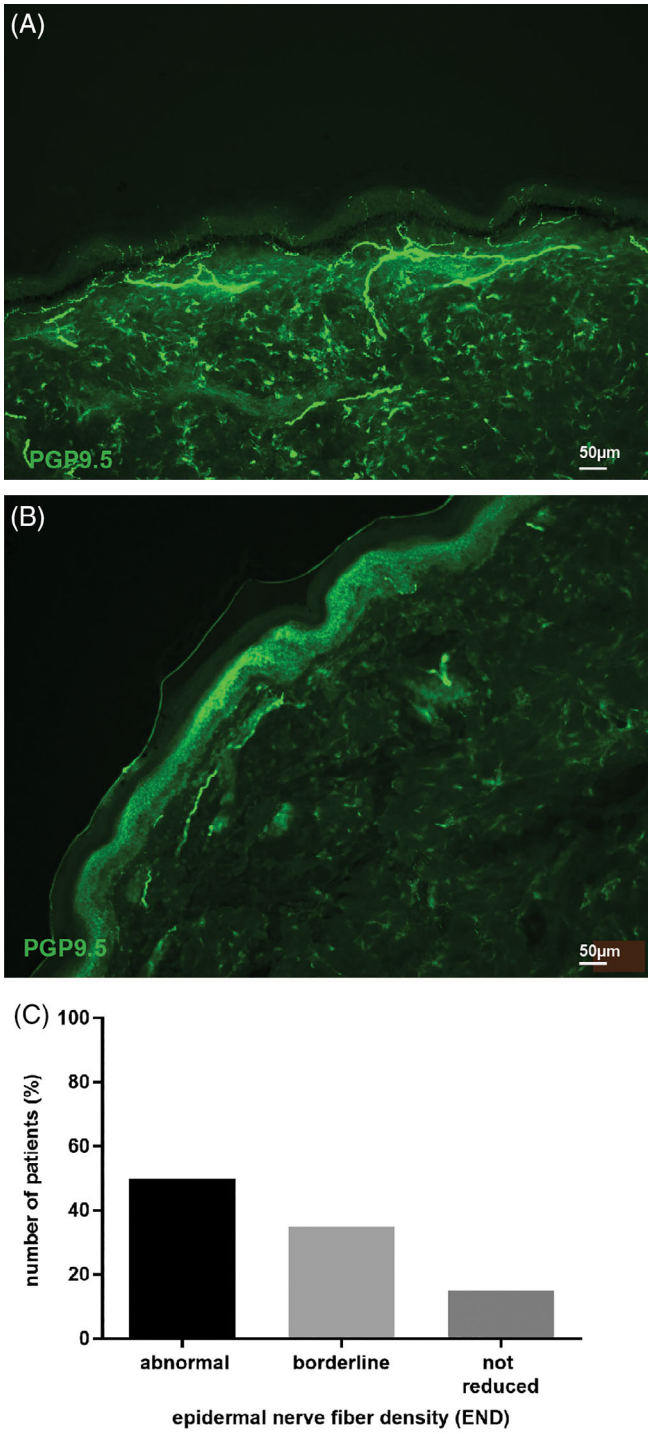

FIGURE 2 END in distal skin biopsies of the pain group. Analysis of END with antibodies against PGP 9.5 in 50-μm sections. Patient 25 with normal END distribution (A), and patient 20 with severe reduction (B). Distribution of END in the pain patients (C). END, epidermal nerve density; PGP 9.5, protein gene product 9.5. Scale bars = 50 μm

detected in a 4-year-old girl with 34.1 fibers/mm, and END showed a negative correlation with age (Spearman's ρ = 0.65; *P* = 0.008; Figure 1B, Table 2). Analysis of SG innervation revealed a mean SND of 46% (36%–57%). Sudomotor neurite density showed no correlation with age (Spearman's ρ = –0.56; *P* = 0.05) or END (Spearman's ρ = 0.47; *P* = 0.12; Figure 1C,D; Table 2).

3.3 | Analysis of END and SND in patients with pain

Patients with pain showed a mean END of 7.2 ± 3.9, varying from 0.7 to 17.6 fibers/mm. Epidermal nerve fiber density was abnormal in 50% and borderline in 35% of patients (Figure 2A-C, Table 3). Epidermal nerve density in samples with abnormal and borderline END was 6.17 ± 2.7 fibers/mm. Sudomotor neurite density could be analyzed in 20 patients. The mean value was 31.35% ± 10.05% (20%–58%) and was abnormal in 55% and borderline in 30% of patients (Table 3). Spearman's rank correlation analysis of SND and END showed no correlation (Spearman's ρ = 0.34; *P* =0.14).

3.4 | Comparison of clinical data with END and SND in patients with pain

In only four patients with pain was END normal: one patient (P19) with hereditary spastic paraplegia (Strümpell Lorrain Syndrome) and three patients (P21, P24, P25) with pain of unknown underlying disease (Table 3, Table S1). The disease progression did not correlate with END or SND (Spearman's ρ = –0.40; *P* = 0.09). In addition, END in patients with distal pain (73%, 6.9 fibers/mm, 0.7–12.9) did not significantly differ from END in patients with generalized and diffuse

TABLE 3 Distal skin biopsy analysis of patients with pain

				END		SND	
Patient	Sex	Progression of pain, y	Age at biopsy, y	Fibers/mm	Level of reduction	%	Level of reduction
P20	G	5	20	4.8	Abnormal	27	Abnormal
P24	B	2	19	12.9	None	ND	ND
P13	B	2	19	7.9	Borderline	36	Borderline
P6	G	4	19	5.4	Abnormal	21	Abnormal
P15	G	1	18	2.8	Abnormal	ND	ND
P22	IG	0.5	17	2.4	Abnormal	ND	ND
P1	G	5	17	6.6	Abnormal	26	Abnormal
P5	G	3	17	6.8	Abnormal	24	Abnormal
P12	IB	0.25	17	8.1	Borderline	33	Borderline
P4	B	1	16	6.3	Borderline	28	Abnormal
P23	B	2	16	5.9	Abnormal	51	None
P14	IB	0.25	16	7.1	Borderline	26	Abnormal
P3	G	5	15	2.1	Abnormal	ND	ND
P16	G	2	15	4.7	Abnormal	33	Borderline
P18	AG	13	15	0.7	Abnormal	36	Borderline
P21	B	NR	15	17.6	None	46	None
P8	B	8	14	5.3	Abnormal	24	Abnormal
P10	G	2	12	6.1	Borderline	ND	ND
P25	G	2	12	13.8	None	58	None
P17	IG	0.5	11	9.4	Borderline	31	Borderline
P19	IB	0.25	10	12	None	32	Borderline
P2	B	4	9	8.9	Borderline	27	Abnormal
P26	B	4	9	7.3	Borderline	23	Abnormal
P9	G	5	8	9.4	Borderline	ND	ND
P11	G	4.5	8	3.9	Abnormal	20	Abnormal
P7	G	3.5	7	8	Abnormal	25	Abnormal

Abbreviations: AG, adolescent girl; B, boy; END, epidermal nerve fiber density; IB, infant boy; IG, infant girl; G, girl; M, man; ND, not done; NR, not reported; P, patient with pain; SND, sudomotor neurite density.

pain symptoms (27%, eight fibers/mm, 2.1–17.6; $P = 0.6$). Epidermal nerve density was abnormal in 63% and borderline in 37% of patients with restless leg syndrome, and SND was abnormal in 75%. In all four patients with erythromelalgia, END and SND were abnormal. Sufficient numbers of SGs were available for analysis in only 50% of patients reporting autonomic symptoms. Sudomotor neurite density was abnormal in 64% and borderline in 31% of patients (Table 3). In one patient with normal END (P19), a borderline SND was observed.

4 | DISCUSSION

Analyses of the skin biopsies showed an abnormal END in 50% of our patients, confirming the diagnosis of SFN. The distribution of age and sex was in line with other studies showing that very young children are less affected.[16,22]

The present study has some limitations. Because clinical data were analyzed retrospectively, pain detection questionnaires for standardized classification of pain symptoms and autonomic function tests were not available, and inclusion and exclusion criteria were not specifically defined. Moreover, the number of skin biopsies analyzed in total as well as skin biopsies with adequate number of SGs was rather small, and normal age-matched controls were not available.

The analysis of proximal skin biopsies of nonpain children who underwent a muscle and skin biopsy for diagnosing a muscular disorder confirmed a negative correlation of END with age.[30,31] In our study, the END of a 16-year-old adolescent girl was in the normal range of reported data showing higher values in proximal compared with distal biopsies.[28,30,31,40] However, from these individual cases, it cannot be determined whether END was affected by their underlying disease. These findings in a small cohort of nonpain children highlight the requirement for age-related standard values in younger patients because of the age-dependent decrease in END during childhood and adolescence.

Because we used 20- to 29-year old individuals as reference values, the prevalence of SFN in the young patients with pain might have been underestimated.

Single studies have previously described SFN and END reduction in young patients with pain symptoms. Only a few children with pain showed a severely reduced END consistent with the findings in our study.[16,21-23,41] In our cohort, the degree of END did not correlate with pain distribution or pain progression. Epidermal nerve fiber density can correlate with disease progression but mainly in conditions that are less frequent in younger patients, such as amyloid neuropathy and diabetic neuropathy.[42-44]

The underlying etiology of SFN is heterogeneous but can be determined in most adult patients. Most common are metabolic diseases such as prediabetes, diabetes mellitus and glucose intolerance, autoimmune diseases, sodium channel gene mutations, toxic, coeliac disease, and vitamin B_{12} deficiency.[2,4-7,45] In children, SFN can occur in genetic disorders including Fabry disease, hereditary sensory autonomic neuropathy-1, and channel mutations and additionally can affect children with immune-mediated, metabolic, or endocrine disorders.[10,46]

In our study, the diagnosis of SFN elicited additional clinical testing and led to the discovery of the presumed underlying etiology in 42% of patients. This included metabolic, hereditary, and autoimmune etiologies. One patient with diabetes mellitus type 1 reported constant neuropathic pain and was first misdiagnosed as psychosomatic. A skin biopsy with severely reduced END confirmed the diagnosis of SFN. Abnormal END in young pain patients with diabetes has also been reported in other studies.[16,21] Although erythromelalgia was been diagnosed in 24% of the patients, genetic testing did not reveal any known pathogenic mutations in the *SCN9A* gene.[47,48] Mutations in *SCN9A*, which codes for a voltage gated sodium channel (Nav1.7), are associated with erythromelalgia, which is characterized by episodic neuropathic pain particularly triggered by warm temperature. The frequency of *SCN9A* mutations in juvenile SFN should be assessed in a larger cohort. A rare pathogenic mutation in the nociceptor TRPA1 (transient receptor potential A1) was found in one patient. This patient had early onset of pain symptoms at 3.5 years of age that were triggered by cold temperature. One patient with such a mutation in whom QST was normal and skin biopsy was not performed was previously reported.[49] However, the finding of abnormal END is in line with other studies describing END reduction in erythromelalgia conditions.[50]

In some patients in our study, laboratory test results provided evidence of an autoimmune mediated disease. This is in line with other studies postulating autoimmune diseases as a major cause of SFN in children, with a benefical clinical effect of immunotherapies.[21,51] In addition, infections can trigger autoimmune neuropathies, and SFN is described in postinfectious conditions.[21,23] This was also seen in our patients. One patient in our study with Lambert Eaton myasthenic syndrome with duplication at Xp11.22-p11.23 reported predominant distal pain triggered by cold temperature.[52] The Xp11.2 duplication might have contributed to the susceptibility of developing an autoimmune disorder because it contains *FOXP3*, which codes for a transcription factor expressed by regulatory T cells.[53] The pain disappeared completely after immunosuppressive treatment. In addition, three patients with hereditary Charcot–Marie–Tooth disease who reported unusually strong pain showed abnormal END. However, painful neuropathies can be associated with an impairment in both small and large nerve fibers.[54,55]

Analysis of SND as a variable to determine the density of autonomic nerve fibers might be helpful, especially when testing for sudomotor function, such as quantitative sudomotor axon reflex test, is not available. However, the analysis of SND in skin biopsies is time consuming and, therefore, difficult to use in routine diagnostics.[37,39] In addition, an adequate number of SGs cannot be obtained in every skin biopsy. In our study, 64% of patients with autonomic disturbance showed an SND reduction. These results underscore the involvement of SND and autonomic dysfunction in young patients with SFN. There was no significant correlation between END and SND, in contrast to

adults with diabetic SFN.[37] A possible explanation could be the small number of biopsies in which SND was analyzed. In one patient with normal END, a borderline SND was observed, indicating that reduction in SND can occur earlier than reduction of END.[36] In the nonpain group, the mean SND of 46% is in line with other studies that have analyzed healthy adults.[37]

In summary, our study demonstrates abnormal END in 50% of young patients with chronic unexplained pain and describes pathological SG innervation in young patients with autonomic symptoms. These findings support the requirement for skin biopsies as a diagnostic tool in a subset of young patients with a pain syndrome and can help uncover an underlying disease that may be causally related and improve clinical care. Additional studies to determine age-related reference values in healthy children and to analyze larger cohorts of young pain patients are required.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest concerning this report.

ETHICAL PUBLICATION STATEMENT

The authors confirm that they have read the Journal's position and issues involved in the ethical publication and affirm that this report is consistent with those guidelines.

REFERENCES

1. Bakkers M, Faber CG, Hoeijmakers JG, Lauria G, Merkies IS. Small fibers, large impact: quality of life in small-fiber neuropathy. *Muscle Nerve*. 2014;49(3):329-336.
2. Devigili G, Tugnoli V, Penza P, et al. The diagnostic criteria for small fibre neuropathy: from symptoms to neuropathology. *Brain*. 2008; 131(Pt 7):1912–1925.
3. Üçeyler N, Vollert J, Broll B, et al. Sensory profiles and skin innervation of patients with painful and painless neuropathies. *Pain*. 2018; 159(9):1867-1876.
4. de Greef BTA, Hoeijmakers JGJ, Gorissen-Brouwers CML, Geerts M, Faber CG, Merkies ISJ. Associated conditions in small fiber neuropathy—a large cohort study and review of the literature. *Eur J Neurol*. 2018;25(2):348-355.
5. Farhad K, Traub R, Ruzhansky KM, Brannagan TH 3rd. Causes of neuropathy in patients referred as "idiopathic neuropathy". *Muscle Nerve*. 2016;53(6):856-861.
6. Lang M, Treister R, Oaklander AL. Diagnostic value of blood tests for occult causes of initially idiopathic small-fiber polyneuropathy. *J Neurol*. 2016;263(12):2515-2527.
7. Üçeyler N. Small fiber pathology—a culprit for many painful disorders? *Pain*. 2016;157(Suppl 1):S60-S66.
8. Lauria G, Lombardi R. Skin biopsy in painful and immune-mediated neuropathies. *J Periph Nerv Syst*. 2012;17(Suppl 3):38-45.
9. Levine TD. Small fiber neuropathy: disease classification beyond pain and burning. *J Cent Nerv Syst Dis*. 2018;10:1179573518771703.
10. Oaklander AL, Nolano M. Scientific advances in and clinical approaches to small-fiber polyneuropathy: a review. *JAMA Neurol*. In press.
11. Cazzato D, Lauria G. Small fibre neuropathy. *Curr Opin Neurol*. 2017; 30(5):490-499.
12. Gemignani F, Giovanelli M, Vitetta F, et al. Non–length dependent small fiber neuropathy. a prospective case series. *J Periph Nerv Syst*. 2010;15(1):57-62.
13. Gorson KC, Herrmann DN, Thiagarajan R, et al. Non–length dependent small fibre neuropathy/ganglionopathy. *J Neurol Neurosurg Psychiatry*. 2008;79(2):163-169.
14. Lawson VH, Grewal J, Hackshaw KV, Mongiovi PC, Stino AM. Fibromyalgia syndrome and small fiber, early or mild sensory polyneuropathy. *Muscle Nerve*. 2018;58(5):625-630.
15. Abad F, Diaz-Gomez NM, Rodriguez I, Perez R, Delgado JA. Subclinical pain and thermal sensory dysfunction in children and adolescents with Type 1 diabetes mellitus. *Diabet Med*. 2002;19(10):827-831.
16. Hoeijmakers JG, Faber CG, Miedema CJ, Merkies IS, Vles JS. Small fiber neuropathy in children: two case reports illustrating the importance of recognition. *Pediatrics*. 2016;138(4). pii: e20161215.
17. Chan AC, Wilder-Smith EP. Small fiber neuropathy: Getting bigger! *Muscle Nerve*. 2016;53(5):671-682.
18. Hopkin RJ, Bissler J, Banikazemi M, et al. Characterization of Fabry disease in 352 pediatric patients in the Fabry Registry. *Pediatr Res*. 2008;64(5):550-555.
19. Burlina AP, Sims KB, Politei JM, et al. Early diagnosis of peripheral nervous system involvement in Fabry disease and treatment of neuropathic pain: the report of an expert panel. *BMC Neurol*. 2011;11:61.
20. Lieber S, Blankenburg M, Apel K, Hirschfeld G, Hernaiz Driever P, Reindl T. Small-fiber neuropathy and pain sensitization in survivors of pediatric acute lymphoblastic leukemia. *Eur J Paediatr Neurol*. 2018; 22(3):457-469.
21. Oaklander AL, Klein MM. Evidence of small-fiber polyneuropathy in unexplained, juvenile-onset, widespread pain syndromes. *Pediatrics*. 2013;131(4):e1091-e1100.
22. Wakamoto H, Hirai A, Manabe K, Hayashi M. Idiopathic small-fiber sensory neuropathy in childhood: a diagnosis based on objective findings on punch skin biopsy specimens. *J Pediatr*. 1999;135(2 Pt 1): 257-260.
23. Kafaie J, Al Balushi A, Kim M, Pestronk A. Clinical and laboratory profiles of idiopathic small fiber neuropathy in children: case series. *J Clin Neuromuscul*. 2017;19(1):31-37.
24. Üçeyler N, Zeller D, Kahn AK, et al. Small fibre pathology in patients with fibromyalgia syndrome. *Brain*. 2013;136(Pt 6):1857–1867.
25. Grayston R, Czanner G, Elhadd K, et al. A systematic review and meta-analysis of the prevalence of small fiber pathology in fibromyalgia: implications for a new paradigm in fibromyalgia etiopathogenesis. *Semin Arthritis Rheum*. 2019;48(5):933-940.
26. Blankenburg M, Boekens H, Hechler T, et al. Reference values for quantitative sensory testing in children and adolescents: developmental and gender differences of somatosensory perception. *Pain*. 2010; 149(1):76-88.
27. Lauria G, Hsieh ST, Johansson O, et al. European Federation of Neurological Societies/Peripheral Nerve Society Guideline on the use of skin biopsy in the diagnosis of small fiber neuropathy. Report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society. *Eur J Neurol*. 2010;17(7):903–912, e944-e909.
28. Provitera V, Gibbons CH, Wendelschafer-Crabb G, et al. A multi-center, multinational age- and gender-adjusted normative dataset for immunofluorescent intraepidermal nerve fiber density at the distal leg. *Eur J Neurol*. 2016;23(2):333-338.

29. Bakkers M, Merkies IS, Lauria G, et al. Intraepidermal nerve fiber density and its application in sarcoidosis. *Neurology*. 2009;73(14):1142-1148.
30. McArthur JC, Stocks EA, Hauer P, Cornblath DR, Griffin JW. Epidermal nerve fiber density: normative reference range and diagnostic efficiency. *Arch Neurol*. 1998;55(12):1513-1520.
31. Panoutsopoulou IG, Luciano CA, Wendelschafer-Crabb G, Hodges JS, Kennedy WR. Epidermal innervation in healthy children and adolescents. *Muscle Nerve*. 2015;51(3):378-384.
32. Lauria G, Bakkers M, Schmitz C, et al. Intraepidermal nerve fiber density at the distal leg: a worldwide normative reference study. *J Peripher Nerv Syst*. 2010;15(3):202-207.
33. Hoeijmakers JG, Faber CG, Lauria G, Merkies IS, Waxman SG. Small-fibre neuropathies—advances in diagnosis, pathophysiology and management. *Nat Rev Neurol*. 2012;8(7):369-379.
34. Kennedy WR, Wendelschafer-Crabb G. Utility of skin biopsy in diabetic neuropathy. *Semin Neurol*. 1996;16(2):163-171.
35. Low VA, Sandroni P, Fealey RD, Low PA. Detection of small-fiber neuropathy by sudomotor testing. *Muscle Nerve*. 2006;34(1):57-61.
36. Dabby R, Djaldetti R, Shahmurov M, et al. Skin biopsy for assessment of autonomic denervation in Parkinson's disease. *J Peripher Nerv Syst*. 2006;113(9):1169-1176.
37. Gibbons CH, Illigens BM, Wang N, Freeman R. Quantification of sweat gland innervation: a clinical-pathologic correlation. *Neurology*. 2009;72(17):1479-1486.
38. Dabby R, Vaknine H, Gilad R, Djaldetti R, Sadeh M. Evaluation of cutaneous autonomic innervation in idiopathic sensory small-fiber neuropathy. *J Peripher Nerv Syst*. 2007;12(2):98-101.
39. Gibbons CH, Illigens BM, Wang N, Freeman R. Quantification of sudomotor innervation: a comparison of three methods. *Muscle Nerve*. 2010;42(1):112-119.
40. Provitera V, Gibbons CH, Wendelschafer-Crabb G, et al. The role of skin biopsy in differentiating small fiber neuropathy from ganglionopathy. *Eur J Neurol*. 2018;25:848-853.
41. Kafaie J, Kim M, Krause E. Small fiber neuropathy following vaccination. *J Clin Neuromuscul Dis*. 2016;18(1):37-40.
42. Divisova S, Vlckova E, Srotova I, et al. Intraepidermal nerve-fibre density as a biomarker of the course of neuropathy in patients with Type 2 diabetes mellitus. *Diabet Med*. 2016;33(5):650-654.
43. Shun CT, Chang YC, Wu HP, et al. Skin denervation in type 2 diabetes: correlations with diabetic duration and functional impairments. *Brain*. 2004;127(Pt 7):1593-1605.
44. Masuda T, Ueda M, Suenaga G, et al. Early skin denervation in hereditary and iatrogenic transthyretin amyloid neuropathy. *Neurology*. 2017;88(23):2192-2197.
45. Lauria G, Merkies IS, Faber CG. Small fibre neuropathy. *Curr Opin Neurol*. 2012;25(5):542-549.
46. Paticoff J, Valovska A, Nedeljkovic SS, Oaklander AL. Defining a treatable cause of erythromelalgia: acute adolescent autoimmune small-fiber axonopathy. *Anesth Analg*. 2007;104(2):438-441.
47. Faber CG, Hoeijmakers JG, Ahn HS, et al. Gain of function Nanu1.7 mutations in idiopathic small fiber neuropathy. *Ann Neurol*. 2012;71(1):26-39.
48. Dib-Hajj SD, Black JA, Waxman SG. NaV1.9: a sodium channel linked to human pain. *Nat Rev Neurosci*. 2015;16(9):511-519.
49. Kremeyer B, Lopera F, Cox JJ, et al. A gain-of-function mutation in TRPA1 causes familial episodic pain syndrome. *Neuron*. 2010;66(5):671-680.
50. Cook-Norris RH, Tollefson MM, Cruz-Inigo AE, Sandroni P, Davis MD, Davis DM. Pediatric erythromelalgia: a retrospective review of 32 cases evaluated at Mayo Clinic over a 37-year period. *J Am Acad Dermatol*. 2012;66(3):416-423.
51. Oaklander AL. Immunotherapy prospects for painful small-fiber sensory neuropathies and ganglionopathies. *Neurotherapeutics*. 2016;13(1):108-117.
52. Verbeek S, Vanakker O, Mercelis R, et al. Lambert-Eaton myasthenic syndrome in a 13-year-old girl with Xp11.22-p11.23 duplication. *Eur J Paediatr Neurol*. 2014;18(3):439-443.
53. Bending D, Ono M. From stability to dynamics: understanding molecular mechanisms of regulatory T cells through Foxp3 transcriptional dynamics. *Clin Exp Immunol*. 2019;197:1-23.
54. Laura M, Hutton EJ, Blake J, et al. Pain and small fiber function in Charcot-Marie-Tooth disease type 1A. *Muscle Nerve*. 2014;50(3):366-371.
55. Tavakoli M, Marshall A, Banka S, et al. Corneal confocal microscopy detects small-fiber neuropathy in Charcot-Marie-Tooth disease type 1A patients. *Muscle Nerve*. 2012;46(5):698-704.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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